

Crassulacean Acid Metabolism in the Epiphyte *Tillandsia usneoides* L. (Spanish Moss)¹

RESPONSES OF CO₂ EXCHANGE TO CONTROLLED ENVIRONMENTAL CONDITIONS

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ABSTRACT

Patterns of CO₂ exchange in Spanish moss under various experimental conditions were measured using an infrared gas analysis system. Plants were collected from a study site in North Carolina and placed in a gas exchange chamber for several days of continuous measurements. No substantial seasonal effects on CO₂ exchange were observed. High rates of nocturnal CO₂ uptake were observed under day/night temperature regimes of 25/10, 25/15, 25/20, 30/20, and 35/20 °C; however, daytime temperatures of 40 °C eliminated nighttime CO₂ uptake and a nighttime temperature of 5 °C eliminated nocturnal CO₂ uptake, regardless of day temperature. Constant chamber conditions also inhibited nocturnal CO₂ uptake. Constant high relative humidity (RH) slightly stimulated CO₂ uptake while low nighttime RH reduced nocturnal CO₂ uptake.

Reductions in daytime irradiance to approximately 25% full sunlight had no effect on CO₂ exchange. Continuous darkness resulted in continuous CO₂ loss by the plants, but a CO₂ exchange pattern similar to normal day/night conditions was observed under constant illumination. High tissue water content inhibited CO₂ uptake. Wetting of the tissue at any time of day or night resulted in net CO₂ loss. Abrupt increases in temperature or decreases in RH resulted in sharp decreases in net CO₂ uptake.

The results indicate that Spanish moss is tolerant of a wide range of temperatures, irradiances, and water contents. They also indicate that high nighttime RH is a prerequisite for high rates of CO₂ uptake.

Because of its epiphytic nature and highly specialized morphology, Spanish moss has interested many investigators. Coutinho (4) first discovered characteristics of CAM³ in Spanish moss in 1969. Since then, others have examined the plant's carbon isotope discrimination ratio (15, 16, 23), O₂ exchange (1), and CO₂ exchange characteristics (10, 14). Recent investigations (14) of *in situ* growth rates, tissue acid fluctuations, and ¹⁴CO₂ uptake rates of Spanish moss have shown that growth and CO₂ uptake were maximal during the warm summer months and minimal during the colder winter months. No high temperature inhibition of CO₂ uptake was observed. Wetting of Spanish moss by rain reduced nocturnal CO₂ uptake rates and stimulated low-level CO₂ uptake

during the day. Tissue water content did not appear to influence CO₂ uptake. Finally, low rates of nocturnal CO₂ uptake were observed under isothermal conditions.

In both the field and under controlled conditions, the tissue water content of Spanish moss tracked changes in atmospheric RH (14, 21). High nighttime RH resulted in water uptake by the plant, and low daytime RH resulted in a loss of water. Twenty-four h net water exchange was nearly always negative, indicating that absorption of liquid water was necessary for the maintenance of high tissue water content in the field.

Unfortunately, it was difficult to compare the results obtained in the above study with those of Kluge *et al.* (10) for the following reasons: ¹⁴CO₂ uptake was measured in the field study (14) while Kluge *et al.* (10) measured net CO₂ exchange; their experimental conditions (constant temperature and RH throughout a day/night cycle) did not realistically approximate field conditions; and they used glasshouse-grown Spanish moss of unstated origin. It was the purpose of this study to examine CO₂ exchange of field-collected Spanish moss in the laboratory, using controlled conditions which approximated those found in the field in North Carolina. Such controlled experiments should help explain the results of the field studies (14).

MATERIALS AND METHODS

Net CO₂ exchange in strands of Spanish moss (*Tillandsia usneoides* L.) sealed in a chamber was monitored continuously during 4- to 6-day intervals with a Beckman IR215 Infrared Gas Analyzer (differential; open system) from June to December 1979. The CO₂ concentration of the incoming air was approximately 360 µl·l⁻¹ and varied up to 50 µl·l⁻¹ on a diurnal basis; however, the changes in CO₂ concentration were too gradual to be detected by the analyzer in the differential mode. Before analysis, the air was dried by condensation and passage through ZnCl₂. Calibrations of the IR gas analyzer were made frequently.

The air stream was humidified by passage through water and its dew point determined with an EG&G Model 880 dew point hygrometer. All tubing in the system was Tygon. Air flowed into the plant chamber at a rate of 500 ml·min⁻¹. The chamber was a double-walled glass column approximately 45 cm long, with an inner diameter of 2.5 cm and a total inner volume of 250 ml. The double wall acted as a water jacket which allowed precise control of chamber air temperatures. Gastra (5) has discussed a potential problem in the use of long, tubular chambers. Calculations based on maximal rates of CO₂ exchange observed in Spanish moss found the problem of linearly changing CO₂ concentration as the air flows over the plant due to CO₂ exchange by the plant to be insignificant given the very low CO₂ exchange rates of Spanish moss.

Lighting was provided by a 400 w multi-vapor high intensity

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³ Abbreviations: CAM, Crassulacean acid metabolism; PPFD, photosynthetic photon flux density.

discharge lamp above and 2 parallel 15 w cool white fluorescent lamps below the chamber. PPFD along the top and bottom of the chamber was measured with a LI-COR LI-185 light meter and a LI-190S quantum sensor. PPFD from above always exceeded that from below, so only the former is reported in any experiment. Maximum PPFD along the top of the chamber in all but the light reduction experiments was $450 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at both ends, increasing to $1,900 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the center of the chamber (hereafter described as $450\text{--}1,900 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Irradiance above and below the chamber was reduced by inserting layers of cheesecloth between the lamps and the chamber.

Ten to 20 "healthy" strands of Spanish moss, approximately 15 to 20 cm long, were collected from the study site (14), detached at their dead bases, wetted to ensure maximum tissue water content, allowed to dry, and placed into the chamber. The strands remained in the chamber 4 to 6 days. After placement in the chamber, control conditions (see below) were maintained for the remainder of the day and night and the following 24 h. The CO_2 exchange pattern obtained in the second 24-h period was utilized for comparison with experimental results. Experimental conditions were imposed on the 3rd day, followed by control conditions again on the 4th. If the results of this control matched those of the previous one, a second experiment was conducted on the 5th day. Using this method, the results of an experimental manipulation could be compared with the response of the same plants to control conditions. Each experiment was repeated at least once with a different set of plants.

Control conditions were: PPFD as discussed above, 25 C and 55% RH day, and 20 C and 95% RH night. These conditions were selected based on the environmental conditions under which maximal CO_2 uptake rates were observed in the field (14). When one environmental condition in the chamber was changed during an experiment, all others were adjusted to remain at control levels. Net CO_2 exchange by the empty chamber was monitored during temperature and dew point changes and never deviated from zero net exchange.

Rates of CO_2 exchange are expressed on a Chl basis since determination of the surface area of Spanish moss was impossible (cross-sectional area varied throughout the plant, and surface scales were often larger than stem or leaf width). One g dry weight of Spanish moss contained approximately 1 mg Chl. This relationship changed only slightly throughout the study (monthly mean mg Chl·g dry weight⁻¹, $n = 3\text{--}4$, from June to December 1979: 1.0, 1.0, 0.8, 1.3, 1.3, 1.3, 1.6). Chl was determined according to the method of Holm (7).

RESULTS

Though absolute rates of CO_2 exchange varied between groups of strands, a CO_2 exchange pattern similar to that of many CAM species (11) always appeared under control conditions. Most CO_2 uptake occurred in the dark period with peak rates early in this period, and little or no CO_2 uptake by the end of the dark period. Immediately following illumination, a sharp burst of CO_2 uptake was observed, which quickly dropped to either no net exchange or to a net CO_2 loss until late afternoon when an uptake of CO_2 was often observed. A sharp burst of CO_2 release followed the light-to-dark transition, which quickly changed to CO_2 uptake.

The variability between different sets of Spanish moss strands was relatively large, especially with regard to maximal CO_2 uptake rates. The variability of CO_2 exchange in the same plant material on a day-to-day basis under control conditions was estimated at the beginning of the study to determine at which level a difference in CO_2 exchange might be attributed to an experimental condition. Four samples of Spanish moss were collected and their CO_2 exchange monitored for 3 to 5 continuous days under control conditions. The greatest observed per cent difference in integrated nocturnal CO_2 uptake between nights was 30%. Therefore, an

observed difference in integrated nocturnal CO_2 uptake between experiment and control of less than 30% was considered too small to be attributed to the imposed experimental condition.

Considering seasonal effects on the response of Spanish moss CO_2 exchange, there was a slight trend of decreasing CO_2 uptake under control conditions in field-collected Spanish moss from the summer to the winter; however, the trend was not significant.

Increases in daytime air temperature of either 5 or 10 C had little or no effect on CO_2 exchange by Spanish moss (Fig. 1, A and B), except for the elimination of late afternoon CO_2 uptake. With a 15 C increase in daytime air temperature, late afternoon CO_2 uptake was replaced by a net loss, and very little CO_2 was fixed during the night, in spite of the maintenance of control conditions throughout the night (Fig. 1C).

No effect on CO_2 exchange was observed when the nighttime air temperature was reduced 5 C from control conditions (Fig. 2A). A 10 C reduction in nocturnal air temperature caused a slight decrease in maximal CO_2 uptake rates, yet the total CO_2 fixed during the night was similar to the control (Fig. 2B). A nighttime temperature of 4 C totally eliminated nocturnal CO_2 uptake (Fig. 2C). A 10 C decrease in day and night temperatures reduced nocturnal CO_2 uptake and stimulated small amounts of daytime CO_2 uptake (Fig. 2D).

When constant nocturnal chamber conditions (20 C, 95% RH) were maintained over a 24-h period, nighttime CO_2 uptake was severely reduced (Fig. 3). Similar results were obtained under constant daytime conditions (25 C, 55% RH; data not shown). High day- and nighttime RH had a slight stimulatory effect on nocturnal CO_2 uptake (Fig. 4A) while low day- and nighttime RH

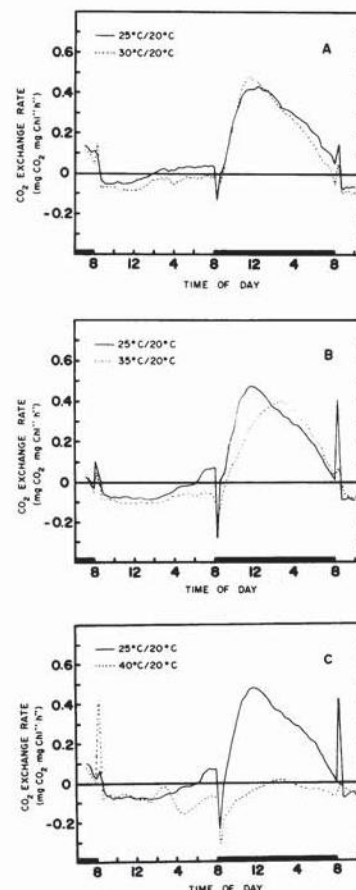


FIG. 1. Effect of increasing daytime air temperature on CO_2 exchange in Spanish moss: 25/20 C (—), 30/20 C (---, A), 35/20 C (---, B), 40/20 C (---, C). (■), darkness. Daytime irradiance was 450 to $1,900 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ across the chamber. RH was 55/95%.

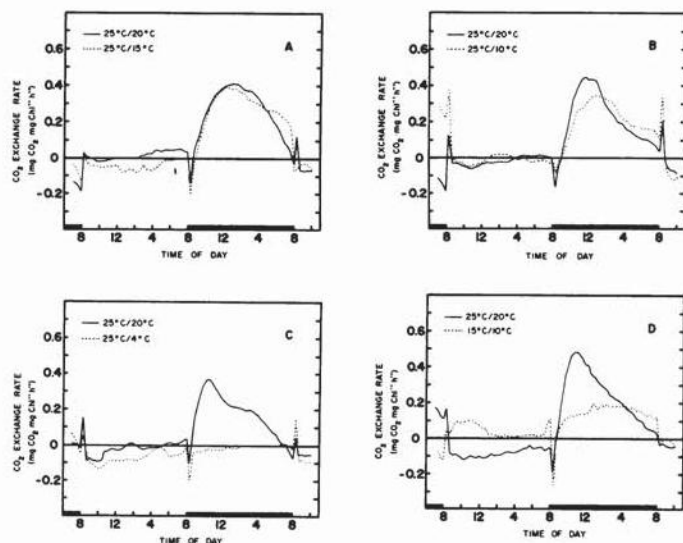


FIG. 2. Effect of decreasing nighttime air temperature on CO₂ exchange in Spanish moss: 25/20 °C (—), 25/15 °C (---), A), 25/10 °C (---, B), 25/4 °C (---, C), 15/10 °C (---, D). (■), darkness. Daytime irradiance was 450 to 1,900 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ across the chamber. RH was 55/95%.

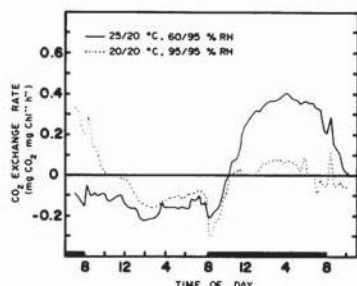


FIG. 3. Effect of constant chamber conditions on CO₂ exchange in Spanish moss: 25/20 °C, 60/95% RH (—); 20/20 °C, 95/95% RH (---). (■), darkness. Daytime irradiance was 450 to 1,900 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ across the chamber.

resulted in a definite decrease in nocturnal CO₂ uptake (Fig. 4B).

A 75% reduction in photosynthetic photon flux density (PPFD) from 450 to 1,900 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (the control conditions across the chamber) to 100 to 400 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, had little effect on CO₂ exchange in Spanish moss. However, under extremely low irradiance conditions (using fluorescent cool-white lamps, 0.1 to 3 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), large amounts of CO₂ were lost throughout the 24-h period (Fig. 5A). Under constant dark conditions, a similar CO₂ loss occurred throughout the 24-h period (Fig. 5B). Under continuous illumination, CO₂ uptake occurred during the normal period of darkness in a fairly typical fashion (data not shown).

The response of CO₂ exchange to wetting the strands of Spanish moss during either the day (Fig. 6A) or the night (Fig. 6B) was the same; net CO₂ loss always resulted. As long as the plant remained wet, no CO₂ uptake occurred.

To determine the effects of different tissue water contents on CO₂ exchange in Spanish moss, strands of Spanish moss were collected from the field after several days of dry weather and placed, without wetting, into the gas exchange chamber. After CO₂ exchange occurred under control conditions for 24 h, the plants were removed, wetted, and placed back into the chamber. The plants were always dry in appearance by the end of the day. The increased tissue hydration resulted in an approximate 40% reduction in nighttime CO₂ uptake (Fig. 7).

An abrupt decrease in air RH during the nighttime CO₂ uptake period resulted in a decrease in the rate of CO₂ uptake (Fig. 8).

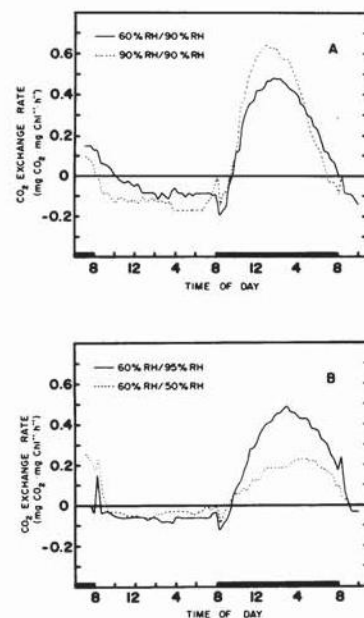


FIG. 4. Effect of high and low nighttime RH on CO₂ exchange in Spanish moss: 60/90% RH (—, A), 60/95% RH (—, B), 90/90% RH (---, A), 60/50% RH (---, B). (■), darkness. Daytime irradiance was 450 to 1,900 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ across the chamber. Air temperature was 25/20 °C.

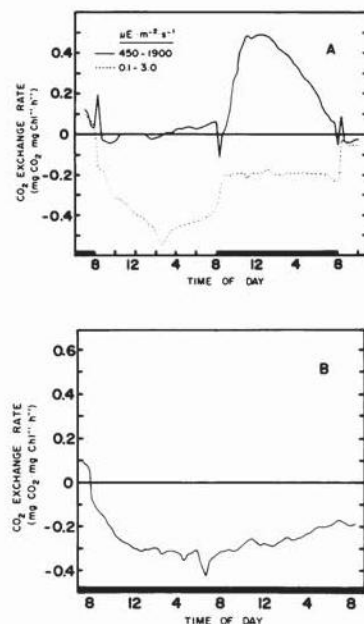


FIG. 5. Effect of different daytime irradiance conditions on CO₂ exchange in Spanish moss: 450 to 1,900 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (—, A), 0.1 to 3.0 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (---, A), 24-h darkness, B. (■), darkness. Air temperature was 25/20 °C. RH was 55/95%.

Similar decreases were noted after abrupt increases in air temperature (with concomitant decreases in RH).

DISCUSSION

The general pattern and rates of CO₂ exchange in *Tillandsia usneoides* L. (Spanish moss) observed throughout this study were similar to those observed with other CAM species (11) and to the patterns obtained by Kluge *et al.* (10) for the same species and Medina *et al.* (15) and Lange and Medina (12) for *Tillandsia*

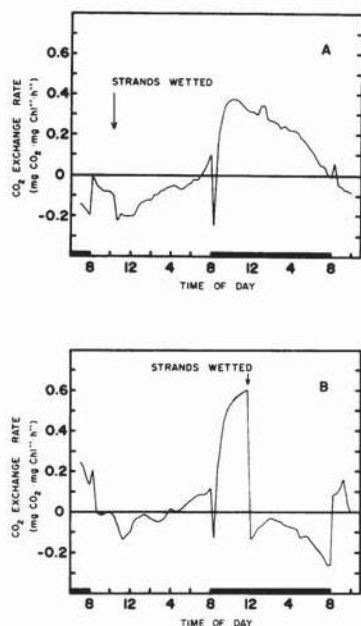


FIG. 6. Effect of wetting on CO_2 exchange in Spanish moss. Strands were removed from the chamber, wetted, and replaced during the day (A) and the night (B). (■), darkness. Daytime irradiance was 450 to $1,900 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ across the chamber. Air temperature was $25/20^\circ\text{C}$. RH was $55/95\%$.

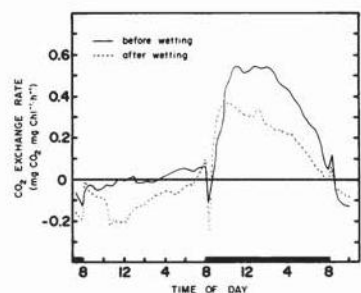


FIG. 7. Effect of tissue hydration on CO_2 exchange in Spanish moss: before wetting (—), after wetting and drying (---). (■), darkness. Daytime irradiance was 450 to $1,900 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ across the chamber. Air temperature was $25/20^\circ\text{C}$. RH was $55/95\%$.

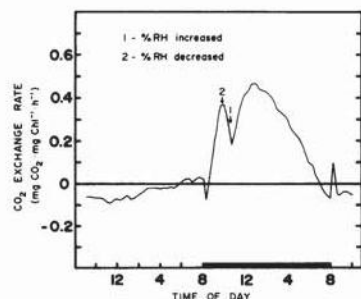


FIG. 8. Effect of abrupt changes in chamber air % RH on CO_2 exchange in Spanish moss. Daytime RH was 55% , nighttime was 92% up to arrow No. 2 (at which point RH was decreased), down to 72% before arrow No. 1 (at which point RH was increased), and 95% the remainder of the night. (■), darkness. Daytime irradiance was 450 to $1,900 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ across the chamber. Air temperature was $25/20^\circ\text{C}$.

recurvata. However, the responses of Spanish moss CO_2 exchange to changing environmental conditions were not always similar.

The results of seasonal measurements of CO_2 uptake for a

North Carolina population of Spanish moss *in situ* showed a decline in gross CO_2 uptake from the warm summer to the cold winter months (14). In the current laboratory study, CO_2 exchange under control conditions in Spanish moss collected from June to December was highly variable. A slight trend of decreasing nocturnal CO_2 uptake was observed, but several samples of Spanish moss collected during the coldest period of the study (November to December) exhibited high nocturnal CO_2 uptake rates, despite the fact that CO_2 uptake in the field at this time was probably zero (14). These results suggest that Spanish moss responded more to the immediate (chamber) conditions than to the past (previous day) conditions.

The temperature response of CO_2 exchange in Spanish moss can be summarized as follows. At least a 5°C difference in day and night temperatures was essential for CO_2 exchange patterns and rates typical for CAM plants. As long as night temperatures were maintained at 20°C , nocturnal CO_2 uptake was unaffected by increased day temperatures up to 35°C . With a constant day temperature of 25°C , decreasing night temperatures had no effect on CO_2 exchange until 5°C was reached. In summary, Spanish moss CO_2 exchange was relatively unaffected by temperature changes until a high or low extreme was reached. At that point, CO_2 uptake was severely reduced.

The temperature effects on CO_2 exchange in Spanish moss described above conflict with those reported by Kluge *et al.* (10) for the same species. Under conditions of constant temperature and RH throughout the day and night, they observed maximal CO_2 uptake rates at 15 and 20°C and inhibition of CO_2 uptake at 25°C and above. The responses to low nighttime temperature (5°C and below) were similar in the two studies. The dissimilar findings of Kluge *et al.* (10) may be attributed to their use of glasshouse-grown Spanish moss and to their use of constant environmental conditions. It is puzzling that they measured any CO_2 uptake by Spanish moss at all under such conditions. Using field-collected plants, little or no CO_2 uptake was found under constant conditions in the current study (at either 20 or 25°C). Inhibition of CAM under isothermal conditions has been reported in other species (3, 24), including Spanish moss under field conditions (14).

Lange *et al.* (13) reported an elimination of daytime CO_2 uptake and enhanced CO_2 release in *Caralluma negevensis* under increasing daytime temperatures. Similarly, late afternoon CO_2 uptake in Spanish moss was eliminated by increasing air temperatures.

High nocturnal CO_2 fixation rates in Spanish moss were observed at nighttime temperatures from 10 to 25°C . These results compare favorably with those of the corresponding field study of $^{14}\text{CO}_2$ uptake in Spanish moss (14). Although a temperature optimum *per se* was not determined for this plant, these results suggest that substantial rates of CO_2 uptake can occur over a fairly broad range of nocturnal temperatures.

The effects of increased day and night air temperatures on nocturnal CO_2 uptake reported above contrast with those reported by Kluge *et al.* (10). They found a reduction in nighttime CO_2 uptake under a constant 25°C regime, and large nocturnal CO_2 losses at 30 and 35°C . Despite these differences, the greatest rates of nighttime CO_2 uptake reported by Kluge *et al.* (10) occurred under 15 and 20°C regimes, within the range of temperatures under which high rates of nocturnal CO_2 uptake were measured in the current study. Medina *et al.* (15) reported maximal rates of nighttime CO_2 uptake for *T. recurvata* and *T. utriculata* at nocturnal temperatures near 15°C .

The inhibition of nocturnal CO_2 assimilation by low nighttime RH in Spanish moss was similar to that reported for the closely related species, *T. recurvata* (12). Lange and Medina (12) attributed the reduced CO_2 uptake rates to an increase in stomatal resistance. Because high nighttime RH results in water uptake by Spanish moss (14, 21), it can be assumed that normal nighttime water uptake did not occur from the air during the night of

decreased RH. Therefore, it seems likely that this nocturnal uptake of atmospheric water is essential for maintenance of high rates of CO₂ uptake in Spanish moss.

No effect on CO₂ exchange of reducing peak irradiance from full sunlight to approximately 25% of full sunlight was observed in the current study. Kluge *et al.* (10) noted a slight reduction in nighttime assimilation of CO₂ as daytime irradiance was decreased from approximately 50 to 10% full sunlight. Reduced CO₂ fixation rates following days of low irradiance have also been reported for other CAM plants (8, 18). The response of Spanish moss CO₂ exchange to decreased irradiance appears to be unique.

The large losses of CO₂ observed throughout the 24-h period under low irradiance were similar to the losses seen under continuous darkness. Previous experiments conducted on CO₂ exchange of CAM plants under conditions of continuous darkness (see references in 11, page 128) have found typical CO₂ exchange patterns during the first 24 h of darkness.

Under continuous illumination, Spanish moss exhibited a CO₂ exchange pattern similar to that observed under control day/night conditions. Similar results have been reported by Kluge (9) and Queiroz (22). Although the biochemical significance of these results is unclear, these studies may provide some insight into the regulatory mechanism of CO₂ exchange in Spanish moss. It seems that light is required to prevent large losses of CO₂ from the tissue during the day, yet darkness is not necessary in order to maintain high rates of CO₂ uptake. These results suggest that the daytime inhibition of CO₂ exchange is externally (environmentally) controlled, while nighttime uptake of CO₂ is internally (enzymically?) controlled.

Despite the fact that Spanish moss took up ¹⁴CO₂ during the day and night when the strands were wet (14), the net CO₂ exchange of wet strands was always negative. On the other hand, Benzing and Renfrow (2) found reduced, yet positive, rates of net CO₂ exchange when two species of *Tillandsia* were wetted. The results of this study of Spanish moss further emphasize the insignificance of daytime uptake of CO₂ as a major source of carbon for the plant.

Kluge *et al.* (10) provided evidence for increased CO₂ uptake in Spanish moss with increasingly desiccated tissue. Similar results were obtained in the current study of Spanish moss. These results are opposite to the findings of most studies with CAM plants, where CO₂ exchange is inhibited by increasing water stress (17, 20, 25). However, it is possible that the strands of Spanish moss were never actually water-stressed in these studies. This problem requires further investigation.

Abrupt increases in temperature and decreases in RH were found to reduce CO₂ uptake rates in Spanish moss and in other CAM plants (12, 19). These responses have been attributed to stomatal regulation of gas exchange and therefore imply that stomata might regulate CO₂ exchange in Spanish moss.

Spanish moss is the most widely distributed member of the Bromeliaceae, occurring from southern Virginia to central Argentina and Chile (6). The results of this study indicate that Spanish moss exhibits wide physiological plasticity in response to different environmental conditions. This wide tolerance of various temperatures, irradiances, and perhaps water contents may allow the plant to colonize many diverse habitats. Low temperatures appear to be a major factor limiting CO₂ exchange and growth in northern and probably southern latitudes. Since the uptake of atmospheric moisture appears necessary for the maintenance of high CO₂ uptake rates, Spanish moss is probably limited in distribution to areas of high nighttime RH. At this North Carolina site, maximum nocturnal RH was seldom less than 90% (14). Furthermore, Spanish moss grows more abundantly in river valleys or near bodies of water as one proceeds inland from the coast (personal observation; and [6]).

In summary, temperature and nighttime RH appeared to be the most influential factors governing CO₂ exchange and growth in Spanish moss. Unlike other CAM plants, high tissue water content inhibited CO₂ uptake in Spanish moss. The results of this study of Spanish moss emphasize the differences between the physiological ecology of terrestrial CAM plants and epiphytic CAM plants.

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